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Reduced photosynthetic thermal acclimation capacity under elevated ozone in poplar (*Populus tremula*) saplings

Running title

Effects of ozone on thermal acclimation

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Abstract

The sensitivity of photosynthesis to temperature has been identified as a key uncertainty for projecting the magnitude of the terrestrial carbon cycle response to future climate change. Although thermal acclimation of photosynthesis under rising temperature has been reported in many tree species, whether tropospheric ozone (O_3) affects the acclimation capacity remains unknown. In this study, temperature responses of photosynthesis (light-saturated rate of photosynthesis (A_{sat}), maximum rates of RuBP carboxylation (V_{cmax}) and electron transport (J_{max})) and dark respiration (R_{dark}) of *Populus tremula* exposed to ambient O_3 (AO$_3$, maximum of 30 ppb) or elevated O_3 (EO$_3$, maximum of 110 ppb) and ambient or elevated temperature (ambient + 5°C) were investigated in solardomes. We found that the optimum temperature of A_{sat} (T_{optA}) significantly increased in response to warming. However, the thermal acclimation capacity was reduced by O_3 exposure, as indicated by decreased T_{optA}, and temperature optima of V_{cmax} (T_{optV}) and J_{max} (T_{optJ}) under EO$_3$. Changes in both stomatal conductance (g_s) and photosynthetic capacity (V_{cmax} and J_{max}) contributed to the shift of T_{optA} by warming and EO$_3$. Neither R_{dark} measured at 25 °C (R_{dark}^{25}) nor the temperature response of R_{dark} were affected by warming, EO$_3$ or their combination. The responses of A_{sat}, V_{cmax} and J_{max} to warming and EO$_3$ were closely correlated with changes in leaf nitrogen (*N*) content and *N* use efficiency. Overall, warming stimulated growth (leaf biomass and tree height), whereas EO$_3$ reduced growth (leaf and woody biomass). The findings indicate that thermal acclimation of A_{sat} may be overestimated if the impact of O_3 pollution is not taken into account.

Keywords: ozone; warming; poplar; photosynthesis; respiration; thermal acclimation; nitrogen; growth

1. Introduction

Global air temperature has increased by 0.74 °C from the preindustrial level, and is predicted to further increase by 1.1-6.4 °C by the end of this century (Collins et al., 2013; IPCC, 2013). As temperature is one of the most important environmental factors controlling physiological processes (e.g. Poethig, 2003; Hegland, Nielsen, Lazaro, Bjerknes, & Totland, 2009), warming is expected to cause shifts in plant functioning that will significantly impact regional ecosystem services (Gauthier, Bernier, Kuuluvainen, Shvidenko, & Schepaschenko, 2015), and global carbon cycling (Graven et al., 2013). However, impacts of warming on tree growth and physiology vary among species. Some studies report that warming reduced (e.g. Way & Sage, 2008; Wertin, McGuire, & Teskey, 2010; Drake et al., 2015), or had no effect (e.g. Cheesman & Winter, 2013; Giguère-Croteau et al., 2019), on tree growth and survival, while others found that warming enhanced forest productivity (e.g. Ghannoum et al., 2010a; Mäenpää, Riikonen, Kontunen-Soppela, Rousi, & Oksanen, 2011; Kasurinen, Biasi, Holopainen, & Oksanen, 2012).

Photosynthesis and respiration regulate plant carbon balance, and thus are key players in the carbon exchange between vegetation and the atmosphere. Plants commonly respond to warming by changes in their photosynthetic and respiratory physiology that act to increase or maintain net carbon gain, which is defined as "thermal acclimation" (Berry & Björkman, 1980; Atkin & Tjoelker, 2003; Way & Yamori, 2014, Drake et al., 2015). Thermal acclimation of the net photosynthetic rate (A_{net}, the rate of carbon fixation minus the loss of CO_2 in respiration) usually occurs via an increase in its temperature optimum (T_{opt}) (Sage & Kubien, 2007; Ghannoum et al., 2010b; Way & Oren, 2010; Crous et al., 2013). Acclimation of respiration rate (R), on the other hand, primarily occurs through a change in the basal respiration rate (i.e. R measured at a given reference temperature), but can also occur via a change in the temperature sensitivity of R (e.g. Atkin & Tjoelker, 2003, Atkin, Bruhn, & Tjoelker, 2005a, Tjoelker, Oleksyn, Lorenc-Plucinska, & Reich, 2009; Crous et al., 2011). These acclimation responses enable vegetation to increase rate of A_{net} and maintain homoeostatic rate of R as climate warming occurs, if the new growth temperature is below T_{opt}.

However, the mechanism of photosynthetic temperature acclimation is still not clear. In general, the temperature response of A_{net} is mainly controlled by changes in stomatal conductance (g_s, the rate of CO_2 entering, or water vapor exiting the leaf through stomata), photosynthetic capacity (the maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase, V_{cmax}, and the maximum rate of electron transport, J_{max}) and respiration (Way & Yamori, 2014). However, it is less clear how the importance of each factor varies among tree species and under different environmental conditions, and what causes this variation. Sensitivity analyses have suggested that all three component processes are equally important in determining the optimum temperature (T_{optA}) of light-saturated rates of photosynthesis (A_{sat}) at the leaf scale (Lin, Medlyn, & Ellsworth, 2012) and at the canopy scale (Tan et al., 2017). However, some contrasting results exist. Based on data from 141 C_3 species, Kumarathunge et al. (2019) reported that the observed global variation in the T_{optA} is primarily explained by biochemical limitations to photosynthesis, rather than by changes in g_s or R. Conversely, Slot and Winter (2017a) found that g_s was a more important limitation of A_{sat} acclimation than leaf biochemistry in four tropical species. Furthermore, Ow, Griffin, Whitehead, Walcroft, and Turnbull (2008) reported that temperature acclimation of A_{sat} was limited, but acclimation of dark respiration (R_{dark}, respiration rate in the absence of light) was apparent in *Populus deltoides* × *nigra* saplings following a transfer to warmer growing conditions. Other studies have also reported limited, or even non-existent thermal acclimation of A_{sat} (e.g. Dillaway & Kruger, 2010; Aspinwall et al., 2017; Benomar et al., 2018). Such variation in observed responses indicates that the mechanisms and the magnitude of photosynthetic temperature acclimation are likely to be species-specific. Although, it has been suggested that net ecosystem productivity may be overestimated if plant physiological adjustments to warming are not accounted for (Smith, Malyshev, Shevliakova, Kattge, & Dukes, 2016), the uncertainties regarding the variation in photosynthetic and respiratory thermal acclimation has hindered their inclusion in global vegetation models (GVMs) that commonly only consider short-term leaf-scale temperature responses.

Much work has recently been carried out on determining the impact of growth temperature on T_{optA} (e.g. Aspinwall et al., 2016; Scafaro et al., 2017; Slot & Winter, 2017b;

Crous et al., 2018; Kumarathunge et al., 2019). Other environmental factors, such as atmospheric CO_2 concentrations (e.g. Ghannoum et al., 2010b; Crous et al., 2013; Kurepin et al., 2018; Dusenge, Madhavji, & Way, 2020), light availability (Niinemets, Oja, & Kull, 1999), and water limitation (Kumarathunge et al., 2020), are also known to both independently affect the T_{optA} and to modify its acclimation response to warming. However, while tropospheric ozone (O_3) is recognized as an important regional air pollutant with strong effects on plant gas exchange (e.g. Ainsworth, Yendrek, Sitch, Collins, & Emberson, 2012; Feng, Wang, Pleijel, Zhu, & Kobayashi, 2016; Dai et al., 2017), its impact on photosynthetic thermal acclimation potential is yet to be determined.

Along with climate warming, O_3 concentrations will continue to rise (e.g. Cooper et al., 2014, Li et al., 2018), and have been increasing from 10-15 ppb in pre-industrial period to ~50 ppb at present in Northern Hemisphere (Cooper et al., 2014; Zhang et al., 2016). There is a positive long-term feedback loop between O_3 and climate warming. Increasing O_3 levels are further accelerating global climate warming (Meehl et al., 2007), while warmer surface air temperatures contribute to increasing O_3 levels (Zeng, Pyle, & Young, 2008). Because O_3 formation is driven by sunlight, the diurnal and seasonal peaks of O_3 usually appear in the afternoon and summer respectively, thus coinciding with the diurnal and seasonal peaks in air temperature. O_3 effects on trees have been widely studied, and O_3 pollution is known to reduce leaf-scale A_{sat} (e.g. Zhang, Feng, Wang, & Niu, 2014; Dai et al., 2017; Xu et al., 2019), increase leaf-scale R (Barnes, 1972; Yang, Skelly, & Chevone, 1983) and reduce the overall tree-scale growth and productivity (e.g. Wittig, Ainsworth, Naidu, Karnoski, & Long, 2009; Li et al., 2017; Feng et al., 2019). However, to date only a few studies have focused on the interactive effects of elevated O_3 and warming on trees. These studies have addressed the responses of the photosynthetic rate (e.g. Mäenpää, Riikonen, Kontunen-Soppela, Rousi, & Oksanen, 2011; Hartikainen et al., 2012; Riikonen et al., 2012), carbon allocation (Kasurinen, Biasi, Holopainen, & Oksanen, 2012) and production of biogenic volatile organic compounds (e.g. Hartikainen et al., 2009, 2012; Kivimäenpää et al., 2012; Ghimire et al., 2016) to factorial combinations of warming and elevated O_3. To our knowledge, no studies have investigated the interactive effect of warming and O_3 exposure on the photosynthetic thermal acclimation capacity. However,

Shang, Xu, Dai, Yuan, and Feng (2019) found that elevated O_3 decreased leaf nitrogen (N) content per area and leaf N allocation to photosynthetic processes, which could decrease leaf Rubisco contents and activity (Evans, 1989). As leaf N reflects total photosynthetic and respiratory enzyme contents (e.g. Reich, Walters, Tjoelker, Vanderklein, & Buschena, 1998; Atkin, Bruhn, & Tjoelker, 2015a) and is closely related to the thermal acclimation of V_{cmax} and A_{sat} (Aspinwall et al., 2017; Scafaro et al., 2017), we speculated that the acclimation potential could be affected by O_3-induced changes in leaf chemistry.

The overall aim of this study was to explore the effects of warming, elevated O_3 and their combination on the thermal acclimation of A_{sat} and R_{dark} in Eurasian aspen (*Populus tremula* L.). This fast-growing broadleaf tree species was chosen for this study due to its extensive planting in the cooler temperate and boreal regions of Europe and Asia. We hypothesized that (1) *Populus tremula* saplings acclimate to warming through adjustments in key leaf gas exchange parameters, including A_{sat}, V_{cmax}, J_{max} and R_{dark} and their temperature responses, especially their optimum temperatures. (2) the thermal acclimation capacity is reduced in *Populus tremula* saplings grown under elevated O_3 compared to those grown under ambient O_3.

2. Materials and methods

2.1 Plant materials

Three-year old poplar (*Populus tremula* L.) saplings were purchased from Cheviot Trees (http://www.cheviot-trees.co.uk/) and planted in 6.5 L pots (diameter 21 cm, height 25 cm) filled with John Innes No.1 compost (J. Arthur Bowers) on 11 April, 2018. The trees were 'bare rooted' and inoculated with mycorrhiza (obtained from Buckingham Nurseries, UK) before planting in pots and watered as required. Trees of similar size (initial average height and stem diameter were 55.3 ± 3.7 cm and 0.43 ± 0.05 cm, respectively) were selected for the experiment. Trees were watered every day to maintain a moist growth medium and were fertilized weekly during the growing season (April-September) with 5.2 g Phostrogen All Purpose Plant Food, equivalent to 50 kg N ha^{-1} over the growing season (Bayer Garden, Cambridge, UK) per pot, in order to prevent nutrient limitations.

2.2 Experimental site and treatments

The experiment was conducted in solardomes (hemispherical glasshouses; 3 m diameter, 2.1 m height) located at Abergwyngregyn Bangor, North Wales, United Kingdom (53.2°N, 4.0°W). After the saplings had acclimated to the solardomes for 20 days, O_3 exposure began on 7 June and finished on 26 September, 2018, when the trees were harvested. There were four treatments each with its own solardome containing six saplings: ambient O_3 (AO$_3$, maximum value, 30 ppb) and elevated O_3 (EO$_3$, maximum value, 110 ppb), combined in a factorial design with ambient temperatures (0T) and ambient + 5°C (5T, warming) treatments. The two O_3 treatments were based on an episodic profile recorded at a rural O_3 monitoring site during July 2006 (Aston Hill, Wales, UK, 52°50'N, 30°3'W). The 5°C increase in temperature reflects the upper end of the Representative Concentration Pathways (RCP8.5) scenarios for land surfaces for 2081-2100 (Collins et al., 2013). The plants and O_3 treatments were rotated among the solardomes every four weeks to minimize any chamber effects.

An O_3 generator (G11, Dryden Aqua, UK) was used to generate O_3 from oxygen concentrated from air (Sequal 10, Pure O_2 Ltd, UK) and the O_3 was distributed to each solardome via PTFE tube and delivered using solenoid valves (Kinesis, UK) controlled by computer software (Lab VIEW version 2012, National Instruments, Texas, US). Charcoal-filtered air was injected with controlled amounts of O_3. In all solardomes, the O_3 concentration was measured for five minutes every 30 minutes using two O_3 analysers (400a, Enviro Technology Services, Stroud, UK; 49i, Thermo Fisher Scientific, Franklin, Massachusetts, USA) of matched calibration. Heating was achieved using air conditioning units (Toshiba Super Digital Inverter, Toshiba-Aircon, UK) that were electronically controlled. In one ambient temperature solardome and two heated solardomes, temperature and relative humidity were continuously measured (Skye Instruments Ltd., Llandrindod Wells, UK) and in one ambient temperature solardome photosynthetically active radiation (PAR) was also continuously monitored (Skye Instruments Ltd., Llandrindod Wells, UK) by automatic weather stations and recorded every five minutes. Climatic conditions and airflow rates were matched between solardomes. During the fumigation period, the 10 h average O_3 concentrations from 08:00 to 18:00 were 23.7 ± 0.7 ppb and 60.2 ± 0.4 ppb for

AO$_3$ and EO$_3$, respectively, 24 h average temperatures were 21.3 ± 0.0 °C and 26.2 ± 0.7 °C for 0T and 5T, respectively. The values of AOT40 (Accumulated hourly O$_3$ concentrations above the threshold of 40 ppb across the daylight hours during the experiments) were 0.5 ppm.h and 47.9 ppm.h for AO$_3$ and EO$_3$, respectively. The profiles of O$_3$ concentrations and temperatures during the experiment are shown in Figure S1.

2.3 Leaf gas exchange

Fully expanded leaves in fifth or sixth leaf position from the top of the plants were chosen for the gas exchange measurements. All gas exchange measurements were carried out on three plants per treatment using a portable open gas exchange system with 6 cm^2 (3 cm x 2 cm) transparent leaf chamber window and LED red and blue light source (Li-6400 XT, LI-COR Inc., Lincoln, NE, USA).

Light-saturated net photosynthesis, stomatal conductance and dark respiration

Starting from August 15, temperature responses of light-saturated rates of photosynthesis (A_{sat}) and stomatal conductance (g_s) were determined at five increasing temperatures (20, 25, 30, 35 and 40 °C) achieved by controlling the block temperature of the leaf cuvette. All leaf gas exchange measurements were completed within seven days. Water jackets (6400-88) were fitted on either side of the sensor head's Peltier coolers to help achieve target measurement temperature within the Li-6400's cuvette using a water bath to control the temperature of water circulating through the jackets. Cuvette conditions included saturating light levels of 1500 µmol m^{-2} s^{-1}, CO$_2$ concentration of 400 µmol mol^{-1} in the air entering the leaf cuvette and flow rate of 500 µmol s^{-1}. Prior to the dark respiration (R_{dark}) measurements, plants were placed in a dark room for at least 30 minutes, which was sufficient for dark acclimation of leaf gas exchange. R_{dark} was measured at 18, 25, 32 and 40 °C respectively under ambient relative humidity, 400 ppm CO$_2$, and a flow rate of 300 µmol s^{-1}.

A/C$_i$ curves

The responses of net assimilation rates to intercellular [CO$_2$] (*A/C$_i$* curves) were

measured on the same plants that were used for determining the temperature responses of A_{sat}, g_s and R_{dark}. These measurements were carried out at saturating light levels of 1500 µmol m^{-2} s^{-1} and a flow rate of 500 µmol s^{-1} across ten $[CO_2]$ steps in the following order: 50, 100, 200, 300, 400, 600, 800, 1000, 1250 and 1500 µmol mol^{-1} at five temperatures (20, 25, 30, 35 and 40 °C). To minimize stomatal closure at high temperatures, leaf chamber relative humidity was controlled between 60% and 80% for each A/C_i curve except at the highest temperature (40°C) where the humidity was about 40% on average. The leaf-to-air vapour pressure deficit (VPD) during these measurements increased with increasing leaf temperature and was consistent at each temperature step, being lower than 3.0 kPa, except at the highest temperature (40 °C) for which it averaged 4.9 kPa (Figure S2).

The CO_2 response curves with empty chamber were measured three times to quantify the chamber CO_2 leakage, and the C_i was recalculated using the average leakage flow (Flexas et al., 2007). From A/C_i curves at each leaf temperature, V_{cmax} (apparent maximum rate of carboxylation of Rubisco) and J_{max} (apparent maximum electron transport rate for RuBP regeneration) were estimated using the 'fitaci' function in R package 'plantecophys' (Duursma, 2015). Although elevated O_3 has been observed to decrease mesophyll conductance (g_m) in poplars (Xu et al., 2019), we did not account for this effect in the current study because curve fitting-based approaches resulted in g_m estimates outside the normally reported range and we had no additional means for estimating g_m as recommended (Pons et al., 2009).

2.4 Fitting temperature responses

The temperature responses of light-saturated rates of net photosynthesis were fitted using a polynomial function (e.g. Robakowski, Li, & Reich, 2012; Crous et al., 2013):

$$A_{sat} = A_{opt} - b \cdot \left(T - T_{opt} \right)^2 \tag{2}$$

Where T_{opt} represents the temperature optimum and A_{opt} is the corresponding A_{sat} at that temperature optimum. The parameter b describes the curvature of the parabola with small b indicating a flat peak (Gunderson, O'Hara, Campion, Walker, & Edwards, 2010).

Because both apparent V_{cmax} and J_{max} peaked within the measured temperature range,

their temperature responses were fitted using a peaked Arrhenius equation (Medlyn et al., 2002):

$$f(T_k) = k_{opt} \frac{H_d \exp(\frac{E_a(T_k - T_{opt})}{T_k R T_{opt}})}{H_d - E_a(1 - \exp(\frac{H_d(T_k - T_{opt})}{T_k R T_{opt}}))}$$

(3)

Where K_{opt} is the value of V_{cmax} or J_{max} at T_{opt} (µmol m^{-2} s^{-1}), H_d is the deactivation energy (kJ mol^{-1}), E_a is the activation energy (kJ mol^{-1}), T_k is the measured leaf temperature (°K), T_{opt} is optimum temperature (°K), and R is the universal gas constant (8.314 J mol^{-1} K^{-1}). To avoid over-parameterization, H_d was held constant at 200 kJ mol^{-1} (Medlyn et al., 2002; Kattge & Knorr, 2007).

The temperature response curve of natural log-transformed R_{dark} (lnR_{dark}) was fitted according to Heskel et al. (2016) as follows:

$$lnR_{dark} = a + b \cdot T + c \cdot T^2$$

(4)

Where a is the value of lnR_{dark} at 0 °C, b is the slope of the temperature response curve of lnR_{dark} at 0 °C, and c is the curvature of the thermal response curve of lnR_{dark}.

The temperature sensitivity of R_{dark} was determined as the Q_{10}, the proportional increase in respiration with a 10 °C increase in temperature.

The Q_{10} for R_{dark} was calculated as:

$$Q_{10} = 10^{10k}$$

(5)

Where k is the slope of the log-transformed respiration rate plotted against leaf temperature (Atkin, Bruhn, & Tjoelker, 2005a).

All of the temperature response curves were fitted in Sigmaplot v.10.0 (Systat software, Chicago, IL, USA).

2.5 Leaf mass per area and nitrogen contents

After the gas exchange measurements, five leaf discs (diameter: 1.9 cm) per leaf used for the photosynthesis measurements were collected and oven-dried at 65 °C for at least 7 days before weighing. Leaf mass per area (LMA, g dry mass m^{-2}) was obtained as the dry

mass of the leaf disc (g) divided by its surface area (m^2). The remaining part of the same leaf was collected and oven-dried at 65 °C for at least 7 days, then ground to a fine powder in a ball mill and used for determining leaf nitrogen (N) content with an elemental analyzer (Vario EL Ⅲ, Elementar, Germany). Nitrogen content per leaf area (N_{area}, g m^{-2}) was obtained from N content per mass and LMA.

2.6 Plant height and biomass

Plant height (cm) was measured and all six trees from each treatment were harvested at the end of the experiment. The biomass was separated into leaves, branches, stems and roots. All plant material was oven-dried at 65 °C for a minimum of 7 days before weighing. The weights of the branches and stem were summed to calculate woody biomass. The weights of the woody biomass and leaves were summed to calculate shoot biomass. Root-to-shoot ratio was calculated as root biomass divided by shoot biomass.

2.7 Statistical analyses

Photosynthetic nitrogen use efficiency (PNUE = A_{sat}/N_{area}), and the ratios of maximum rates of RuBP carboxylation (V_{cmax}/N_{area}) and maximum electron transport rates (J_{max}/N_{area}) at 25 ˚C to leaf nitrogen were calculated from A_{sat}, V_{cmax} and J_{max} and N_{area}, respectively. All data are reported as means ± SE. The data were checked for a normal distribution and homogeneity of variance (Levene's test) before significance testing and log-transformed when necessary. Two-way ANOVAs were used to analyze the main effects of growth temperature and O_3, and their interactions, on temperature response parameters of gas exchange, growth traits and leaf biochemical traits. Simple effects analyses were carried out to determine the main effects when significant interactions were detected. Post-hoc testing of the O_3 and temperature responses was done using Tukey's honest significance test. Differences with $P < 0.05$ were considered statistically significant. All analyses were performed in JMP software (SAS Institute, Cary, NC, USA).

3. Results

3.1 Temperature responses of net photosynthesis, dark respiration and stomatal conductance

The temperature response of A_{sat} was altered by both the warming and EO$_3$ treatments (Figure 1a; Table 1). Both A_{sat}^{25} and A_{opt} were significantly decreased under EO$_3$ by 55.9% and 55.8%, respectively. However, warming had no significant effect on either A_{sat}^{25} or A_{opt} in either O$_3$ treatment. The T_{optA} increased with warming (0.6 °C per 1 °C warming across O$_3$ treatments, i.e. regardless of the O$_3$ concentration), but was significantly decreased by 9.8% under EO$_3$, indicating that both warming and EO$_3$ treatments induced a shift of T_{optA}. EO$_3$ exposure under simultaneous warming (EO$_3$ 5T) resulted in a considerable flattening of the temperature response, b was reduced by 77.8% compared to warming alone (AO$_3$ 5T) (Figure 1a; Table 1). However, no statistically significant interactions between O$_3$ and warming were detected for the temperature response of light-saturated net photosynthesis (Table 1).

R_{dark} increased exponentially with leaf temperature as expected (Figure 1b). There was no effect of EO$_3$, warming or their interaction on R_{dark}^{25} or Q_{10}, indicating that neither of the two treatments affected the temperature sensitivity of R_{dark} (Figure 1b; Table 1).

The temperature responses of g_s were inconsistent among the four treatments (Figure 1c). Warming stimulated g_s^{25} at AO$_3$ by 78.9%, while the combination of warming and EO$_3$ (EO$_3$ 5T) resulted in a 72.9% decrease in g_s^{25} compared to g_s^{25} under warming only (AO$_3$ 5T), indicating a significant interaction of O$_3$ and warming. EO$_3$ had no effect on g_s^{25} at 0T (Figure 1c; Table 1).
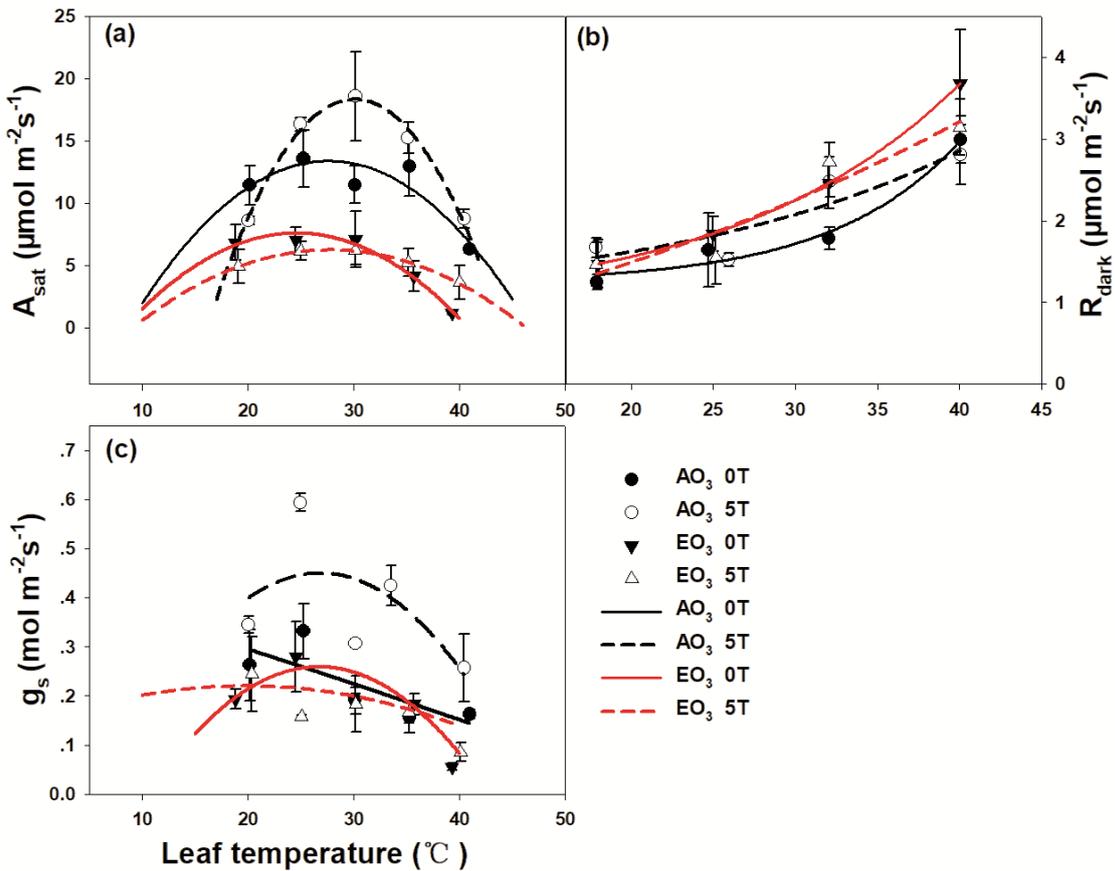
Figure 1. Temperature responses of (a) light-saturated rate of photosynthesis (A_{sat}), (b) dark respiration (R_{dark}) and (c) stomatal conductance (g_s) in poplar saplings exposed to 30 or 110 ppb O_3 at ambient or elevated temperature (ambient + 5°C), respectively. AO_3 0T = 30 ppb O_3 + ambient temperature (black solid line); AO_3 5T = 30 ppb O_3 + warming (black dotted line); EO_3 0T = 110 ppb O_3 + ambient temperature (red solid line); EO_3 5T = 110 ppb O_3 + warming (red dotted line). Means ± SE are shown (n = 3).

Table 1. Effects of growth temperature and O_3 exposure on the temperature responses of light-saturated photosynthesis (A_{sat}^{25} = the photosynthesis rate at 25 °C; A_{opt} = the photosynthesis rate at the temperature optimum; T_{optA} = the temperature optimum of A_{sat}; b = the broadness of the temperature response curve), dark respiration (R_{dark}^{25} = dark respiration measured at 25 °C; Q_{10} = the proportional increase in R_{dark} with a 10 °C increase in temperature), and stomatal conductance (g_s^{25} = stomatal conductance at 25 °C). AO$_3$ 0T = 30 ppb O_3 + ambient temperature; AO$_3$ 5T = 30 ppb O_3 + warming; EO$_3$ 0T = 110 ppb O_3 + ambient temperature; EO$_3$ 5T = 110 ppb O_3 + warming. Means ± SE (n = 3) are shown to the left and a summary report of the two-way ANOVA to the right. Lettering represents statistical differences among treatments based on post hoc testing ($P < 0.05$, Tukey's honest significance test) and bold numbers indicate significant overall ANOVA results. Simple effects analyses were carried out to determine the main effects when significant interactions were detected.

Variables	Unit	AO$_3$ 0T	AO$_3$ 5T	EO$_3$ 0T	EO$_3$ 5T	O$_3$	T$_{growth}$	O$_3$ x T$_{growth}$
A_{sat}^{25}	μmol m^{-2} s^{-1}	13.6 ± 2.3a	16.4 ± 0.5a	7.0 ± 1.0b	6.2 ± 0.8b	**0.0002**	0.492	0.210
A_{opt}	μmol m^{-2} s^{-1}	13.4 ± 2.2ab	18.4 ± 1.8a	7.7 ± 1.3b	6.3 ± 1.0b	**0.0006**	0.306	0.086
T_{optA}	°C	27.4 ± 0.4b	30.2 ± 0.4a	24.2 ± 1.0c	27.7 ± 0.1ab	**0.001**	**0.0005**	0.586
b	-	0.04 ± 0.01ab	0.09 ± 0.03a	0.03 ± 0.00ab	0.02 ± 0.00b	**0.022**	0.183	0.052
R_{dark}^{25}	μmol m^{-2} s^{-1}	1.64 ± 0.45a	1.52 ± 0.08a	1.84 ± 0.21a	1.54 ± 0.32a	0.724	0.508	0.775
Q_{10}	-	1.46 ± 0.04a	1.31 ± 0.06a	1.52 ± 0.08a	1.52 ± 0.21a	0.288	0.548	0.574
g_s^{25}	mol m^{-2} s^{-1}	0.33 ± 0.06b	0.59 ± 0.02a	0.28 ± 0.07b	0.16 ± 0.00b	-	-	**0.003**
						0.603 (0T)	**0.011** (AO$_3$)	
						< 0.0001 (5T)	0.161 (EO$_3$)	

3.2 Temperature response of photosynthetic capacity

The temperature response of V_{cmax} was altered by both warming and EO_3 (Figure 2a; Table 2). The V_{cmax}^{25} was unaffected by warming but was significantly reduced by EO_3 (-59.9%). The optimum temperature of V_{cmax} (T_{optV}) acclimated positively to warming (0.62 °C and 0.2 °C increase per 1 °C of warming for AO_3 and EO_3, respectively). However, T_{optV} was significantly decreased, by 1.6 °C, by EO_3 across the temperature treatments, i.e. regardless of the temperature treatment. Values of V_{cmax} at its thermal optimum (K_{optV}) were significantly decreased at EO_3 by 55.8% and 76.0% for 0T and 5T, respectively. Warming increased K_{optV} by 28.1% at AO_3, but had no effect on K_{optV} at EO_3, reflecting a significant interaction between warming and O_3 (Table 2). The activation energy of V_{cmax} (E_{aV}) was not altered by warming, but EO_3 significantly decreased E_{aV} by 21.6% (Figure 2a; Table 2).

The temperature response of J_{max} was also altered by both warming and EO_3 (Figure 2b; Table 2). Similarly to V_{cmax}^{25}, the J_{max}^{25} was unaffected by warming but was significantly reduced by EO_3. The optimum temperature of J_{max} (T_{optJ}) was not altered by warming in either O_3 treatment, indicating that the T_{optJ} did not acclimate to warming. However, EO_3 significantly decreased T_{optJ} by 3.3 °C across the temperature treatments. Values of J_{max} at its thermal optimum (K_{optJ}) were significantly decreased by 58.6% and 61.4% at EO_3 for 0T and 5T, respectively. Warming reduced K_{optJ} by 12.4% across the O_3 treatments. There was a significant interaction between O_3 and warming on the activation energy of J_{max} (E_{aJ}), which resulted in a reduction of E_{aJ} by EO_3 at 0T, but not at 5T (Table 2).

The J_{max}/V_{cmax} ratio decreased with leaf temperature and neither warming nor EO_3 altered its temperature response (Figure 2c). Furthermore, neither warming nor EO_3 had an effect on J_{max}/V_{cmax} ratio measured at 25 °C ($J_{max}^{25}/V_{cmax}^{25}$) (Table 2).
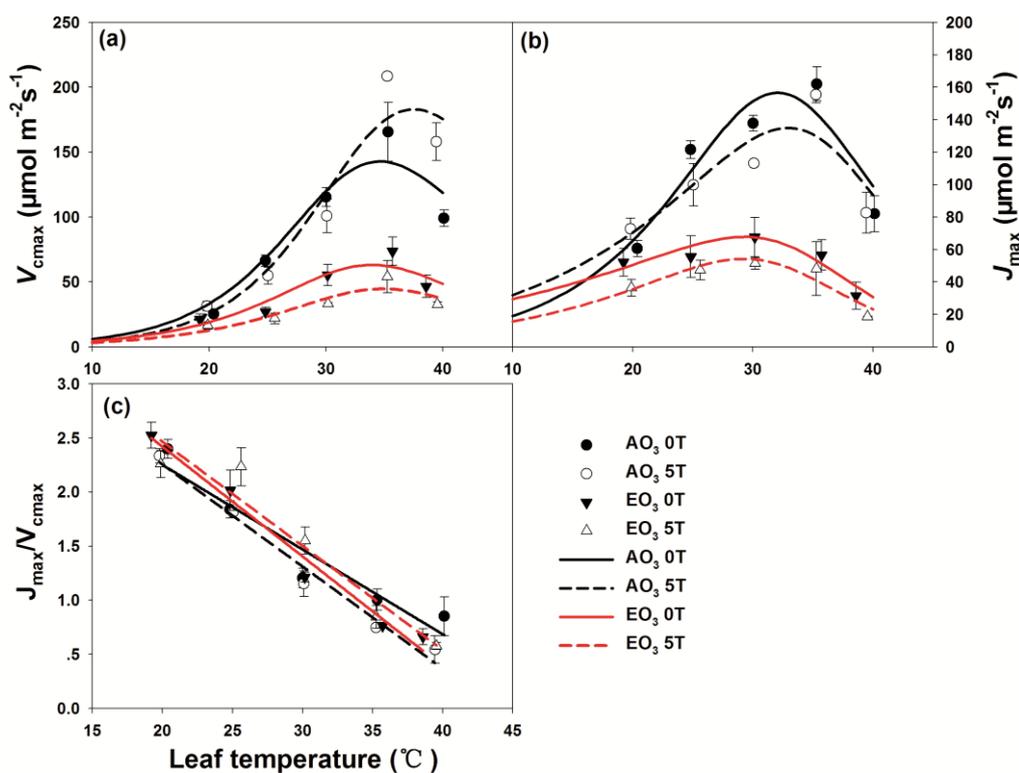
Figure 2. Temperature responses of (a) V_{cmax}: maximum rate of carboxylation of Rubisco; (b) J_{max}: maximum electron transport rate for RuBP regeneration, and (c) J_{max}/V_{cmax} ratio in poplar saplings exposed to 30 and 110 ppb O_3 at ambient and elevated temperature (ambient + 5°C), respectively. AO_3 0T = 30 ppb O_3 + ambient temperature (black solid line); AO_3 5T = 30 ppb O_3 + warming (black dotted line); EO_3 0T = 110 ppb O_3 + ambient temperature (red solid line); EO_3 5T = 110 ppb O_3 + warming (red dotted line). Means ± SE are shown (n = 3).

Table 2. Photosynthetic capacity traits measured (V_{cmax}^{25}, J_{max}^{25} and $J_{max}^{25}/V_{cmax}^{25}$) or fitted ($T_{opt}$, K_{opt} and E_a) from temperature response for poplar (*Populus tremula*) grown at ambient and warming (ambient + 5°C) and exposed to 30 and 110 ppb O_3, respectively. V_{cmax}^{25}, the maximum carboxylation rate at 25 °C, J_{max}^{25}, the maximum electron transport rate at 25 °C, T_{optV} or T_{optJ}, the temperature optimum of V_{cmax} or J_{max}- temperature response, K_{optV} or K_{optJ}, optimum V_{cmax} or J_{max} at the optimum temperature, E_{aV} or E_{aJ}, the activation energy of V_{cmax} or J_{max}-temperature response, $J_{max}^{25}/V_{cmax}^{25}$, the ratio between J_{max}^{25} and V_{cmax}^{25}. AO$_3$ 0T = 30 ppb O_3 + ambient temperature; AO$_3$ 5T = 30 ppb O_3 + warming; EO$_3$ 0T = 110 ppb O_3 + ambient temperature; EO$_3$ 5T = 110 ppb O_3 + warming. The small letters represent statistical differences between treatments (mean ± SE, n = 3) ($P < 0.05$, Tukey's honest significance test). Simple effects analyses were carried out to determine the main effects when significant interactions were detected.

Variables		Unit	AO$_3$ 0T	AO$_3$ 5T	EO$_3$ 0T	EO$_3$ 5T	O$_3$	T$_{growth}$	O$_3$ x T$_{growth}$
Rubisco	V_{cmax}^{25}	µmol m^{-2} s^{-1}	66.3 ± 4.5a	54.9 ± 6.6a	26.9 ± 3.7b	21.8 ± 4.1b	**<0.0001**	0.125	0.527
carboxylation	T_{optV}	°C	34.6 ± 0.2b	37.5 ± 0.5a	33.9 ± 0.7b	34.9 ± 0.5b	**0.014**	**0.007**	0.108
	K_{optV}	µmol m^{-2} s^{-1}	142.9 ± 8.7b	183.0 ± 9.1a	63.1 ± 3.9c	44.0 ± 5.7c	**-**	**-**	**0.004**
							0.001 (0T)	**0.034** (AO$_3$)	
							0.0002 (5T)	0.055 (EO$_3$)	
	E_{aV}	KJ mol^{-1}	120.4 ± 4.9a	128.1 ± 5.8a	100.4 ± 5.6a	94.5 ± 12.8a	**0.010**	0.918	0.415
Electron	J_{max}^{25}	µmol m^{-2} s^{-1}	126.1 ± 6.0a	102.9 ± 13.8ab	56.4 ± 13.3bc	47.9 ± 6.1c	**0.0003**	0.168	0.504
transport	T_{optJ}	°C	32.1 ± 0.9a	33.0 ± 0.6a	29.3 ± 0.9a	29.2 ± 1.0a	**0.005**	0.629	0.578
	K_{optJ}	µmol m^{-2} s^{-1}	163.7 ± 2.2a	140.5 ± 0.7b	67.8 ± 6.7c	54.3 ± 6.6c	**<0.0001**	**0.005**	0.348
	E_{aJ}	KJ mol^{-1}	86.0 ± 4.4a	58.2 ± 11.2ab	38.7 ± 7.2b	58.8 ± 6.5ab	**-**	-	**0.015**

						0.005 (0T)	0.083 (AO_3)	
						0.965 (5T)	0.106 (EO_3)	
$J_{max}^{25}/V_{cmax}^{25}$	-	$1.91 \pm 0.08a$	$1.87 \pm 0.03a$	$2.05 \pm 0.2a$	$2.26 \pm 0.17a$	0.093	0.555	0.383

3.3 Effects of warming and EO_3 on leaf nitrogen and the ratio of photosynthesis parameters to leaf nitrogen

Leaf N_{area} was positively correlated with A_{sat} and photosynthetic capacity (V_{cmax}^{25} and J_{max}^{25}), but was unrelated to R_{dark}, when evaluated across the treatments (Figure S3). Warming had no effects on N_{area} or the efficiency with which N was used for photosynthesis (Figure 3, Table S1). Elevated O_3 decreased N_{area} (-26.6%, ANOVA: $P < 0.0001$), PNUE (-38.8%, ANOVA: $P = 0.010$), V_{cmax}/N (-43.9%, ANOVA: $P = 0.005$), and J_{max}/N (-35.7%, ANOVA: $P = 0.022$). No significant interactions between warming and EO_3 were detected for N_{area} or the nitrogen use efficiency values (Figure 3, Table S1).
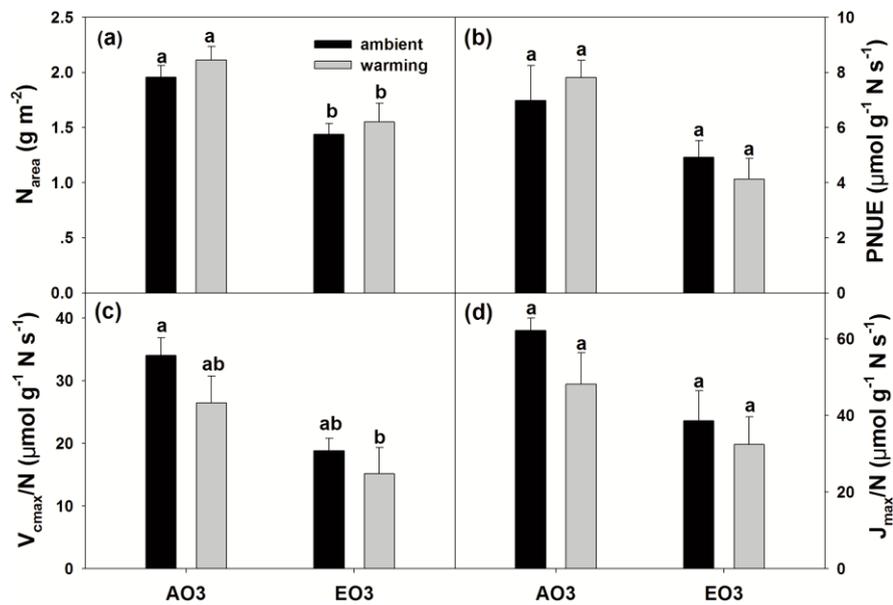

Figure 3. Effects of warming and EO_3 on (a) leaf nitrogen per area (N_{area}), (b) photosynthetic nitrogen use efficiency (PNUE), and the ratios of (c) maximum carboxylation rate of Rubisco (V_{cmax}/N) and (d) maximum electron transport rate (J_{max}/N) at 25 ˚C to leaf nitrogen, respectively. AO_3 = 30ppb O_3, EO_3 = 110ppb O_3. The small letters represent statistical differences between treatments (mean ± SE, n = 6 for N_{area}, n = 3 for PNUE, V_{cmax}/N and J_{max}/N) ($P < 0.05$, Tukey's honest significance test).

3.4 Effects of warming and EO_3 on biomass, leaf mass per area and height

Warming significantly increased leaf biomass (+7.0%, ANOVA: $P = 0.022$) but did not affect woody, stem, root, total biomass or the root-to-shoot ratio. EO_3 significantly

decreased leaf (-7.5%, ANOVA: $P = 0.0099$) and woody (-9.7%, ANOVA: $P = 0.0003$) biomass. However, EO_3 had no significant effects on stem, total, root biomass or the root-to-shoot ratio. There were no significant interactions between warming and O_3 on the biomass parameters (Figure 4a-f, Table S1).

There was no impact of warming on leaf mass per area (LMA) in either O_3 treatment, however, EO_3 significantly decreased LMA by 20.8% (ANOVA: $P < 0.0001$). The interactive effects of warming and O_3 on LMA were not significant (Figure 4g, Table S1). Warming significantly increased sapling height by 8.6% (ANOVA: $P < 0.0001$), while no significant EO_3 effect on height growth were detected (Figure 4h, Table S1).
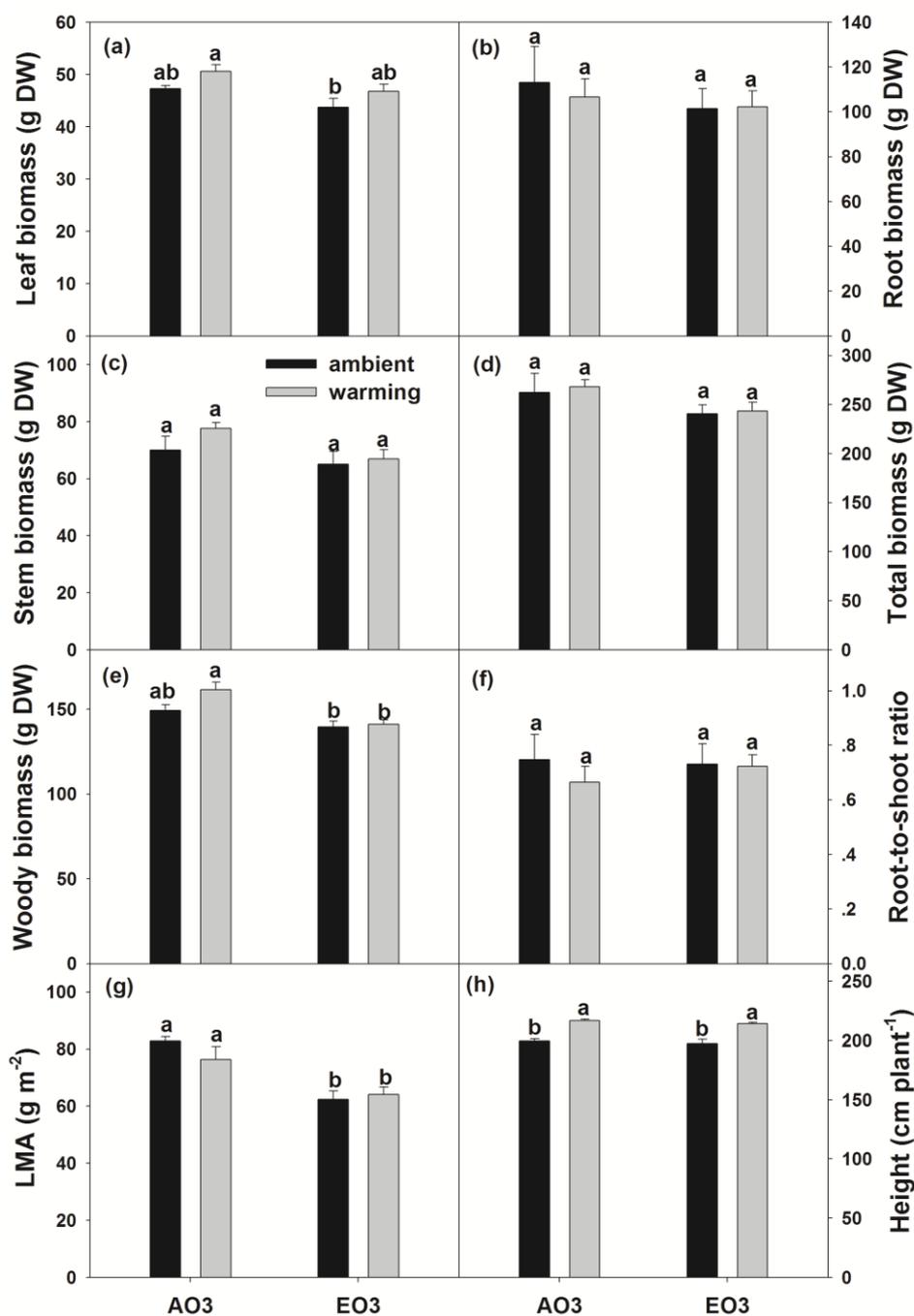
Figure 4. Effects of warming and EO$_3$ on (a) leaf, (b) stem, (c) woody, (d) root, (e) total dry biomass, (f) root-to-shoot ratio, (g) leaf mass per area (LMA) and (h) height of poplar saplings exposed to 30 and 110 ppb O$_3$ at ambient and elevated temperature (ambient + 5°C), respectively. AO$_3$ = 30 ppb O$_3$, EO$_3$ = 110 ppb O$_3$. DW = dry weight. The small letters represent statistical differences between treatments (mean ± SE, n = 6) ($P < 0.05$, Tukey's honest significance test).

4 Discussion

4.1 Effects of warming and EO₃ on the temperature response of light-saturated net photosynthesis and growth

Both the warming and EO₃ treatments influenced the thermal acclimation of A_{sat} in the studied *Populus tremula* saplings (Figure 1a; Table 1). The observed shift in T_{optA}, 0.6 °C per 1 °C warming in AO₃, is consistent with previously reported values that typically are in the range of 0.3-0.5 °C per °C change in growth temperature (Berry & Björkman, 1980; Battaglia, Beadle, & Loughhead, 1996). However, even greater T_{optA} shifts have been reported (e.g. Sendall et al., 2015; Dusenge, Madhavji, & Way, 2020), suggesting that considerable differences exist in the physiological capacities for photosynthetic thermal acclimation among trees species. In addition, variations in the degree and duration of temperature treatments and in the surrounding environmental factors likely explain some of the reported differences. Despite the change in T_{optA}, the light-saturated photosynthetic rates, A_{sat}^{25} and A_{opt}, of the studied saplings were unaffected by the warming treatment. This type of constructive acclimation response helps plants to cope with changes in their growth environment by leading to an enhancement or maintenance of carbon uptake (Way & Yamori, 2014). Here we found that warming resulted in larger leaf biomass and plant height (Figure 4) indicating that poplars growing in Northern Wales are currently temperature-limited, able to thermally acclimate and that moderate warming is likely to benefit their long-term productivity. In addition, as climate change is predicted to be accompanied by increased frequency of extreme heat events (IPCC, 2013), an increase in T_{optA} and maintenance of A_{sat} at higher growth temperatures may also enhance the survival of poplar via improved carbon gain compared to plants with weaker acclimation capacity during periods of high thermal stress (Dusenge, Madhavji, & Way, 2020).

In contrast, exposure to elevated O₃ resulted in a detractive adjustment of A_{sat} through significantly decreased T_{optA} and A_{opt} (Figure 1; Table 1), leading to reduced woody and leaf biomass, and LMA in the EO₃-exposed saplings (Figure 4). The reduction of A_{sat} at EO₃ is common, and has been reported for many tree species, for example *Cyclobalanopsis glauca* (Zhang, Feng, Wang, & Niu, 2014), *Prunus persica* (Dai et al., 2017) and *Populus* (e.g. Shang et al., 2017; Xu et al., 2019). The reduction in T_{optA} by EO₃ observed here has

not been reported previously. We saw no evidence of EO_3 affecting the thermal acclimation capacity of A_{sat} *per se*, as no growth temperature x O_3 interaction was observed on A_{sat}^{25}, A_{opt} or T_{optA} (Table 1). However, our findings do suggest that reduced T_{optA} under elevated O_3 may increase the risk for severe photosynthetic inhibition and productivity loss in response to thermal stress in O_3-polluted regions. While the impact of EO_3 on photosynthetic thermal acclimation has received little previous attention, negative effects of elevated O_3 on tree growth are commonly reported. For example, a quantitative meta-analysis indicated that ambient O_3 concentrations with an average of 40 ppb significantly decrease total tree biomass by 7% compared to preindustrial O_3 levels in the northern hemisphere (approximately 10 ppb) (Wittig, Ainsworth, Naidu, Karnoski, & Long, 2009). Furthermore, another meta-analysis reported that elevated mean O_3 of 116 ppb reduced total biomass of woody plants by 14% compared with the control (mean O_3 of 21 ppb; Li et al., 2017). In general, the reported impacts of EO_3 on biomass allocation are highly variable within and among tree species. We found that EO_3 significantly reduced woody biomass, but had no effect on root biomass and root-to-shoot ratio (Figure 4). Shang et al. (2017) and Li et al. (2020) who also studied poplar reported that EO_3 had a greater impact on root than on aboveground biomass, and that EO_3 did not impact root-to-shoot ratio, respectively. The differences in the observed biomass allocation responses to elevated O_3 likely result from differences in O_3 exposure characteristics among the experiments, interspecies variations, ontogenetic changes and interacting environmental conditions.

4.2 Mechanisms underlying the thermal acclimation responses of net photosynthesis

The main factors controlling the temperature response of net photosynthesis include stomatal conductance, photosynthetic capacity and respiration (Lin, Medlyn, & Ellsworth, 2012; Way & Yamori, 2014). In the present study, warming significantly increased g_s^{25}, while EO_3 reduced it, with a significant interaction between the two leading to reduced g_s^{25} at high growth temperature under EO_3 (Table 1). Warming-induced g_s enhancement may contribute to leaf cooling through an increase in transpiration rate, and therefore protects leaves from high temperature damage and photosynthetic inhibition (Slot & Winter, 2017b; Urban, Ingwers, McGuire, & Teskey, 2017; Drake et al., 2018). It may also help in

achieving or maintaining high photosynthetic rates by facilitating the diffusion of CO_2 to the sites of carboxylation. However, other studies have found that warming decreases g_s, for example, in European aspen (Mäenpää, Riikonen, Kontunen-Soppela, Rousi, & Oksanen, 2011) and different spruce species (Lamba et al., 2018; Dusenge, Madhavji, & Way, 2020). This has been suggested to occur to reduce water loss by transpiration in order to protect the integrity of the hydraulic system during periods of low soil water availability and high driving force for transpiration, i.e. high vapor pressure deficit due to high temperature (Oren et al., 1999; Marchin et al., 2016). Overall, stomatal response to warming will depend on interactions among multiple factors such as the degree of warming, species and soil moisture contents (Lin, Medlyn, & Ellsworth, 2012; Marchin et al., 2016). In our study, to evaluate maximum potential treatments responses, saplings were grown with non-limiting soil moisture and nutrients, therefore their responses were not constrained by low soil moisture or reduced nutrient uptake, which may occur under periods of high temperatures under natural growth conditions (Ghannoum et al., 2010a).

Reduction of g_s by EO_3 is commonly reported for many tree species (e.g. Dai et al., 2017; Li et al., 2017). Ozone exposure commonly leads to greater decrease in A_{sat} than in g_s, leading to increased CO_2 concentration in the leaf intercellular space (C_i), and decreased gas-phase limitations of A_{sat} (Wittig, Ainsworth, & Long, 2007). This decoupling of photosynthetic and stomatal regulation under elevated O_3 may lead to stomatal sluggishness (Hoshika, Carriero, Feng, Zhang, & Paoletti, 2014). Here EO_3-induced a strong g_s decrease in the high temperature treatment, while no EO_3 effect on g_s was observed under ambient temperature. This suggests that simultaneous periods of high temperatures and high O_3 concentrations may interact to compromise the thermal acclimation capacity, the ability to reduce leaf temperatures by transpiration and the ability ensure sufficient CO_2 supply for photosynthesis in poplars. These findings are partly supported by the results of Slot & Winter (2017a), who reported that g_s is a key limitation of the thermal acclimation of net photosynthesis compared to biochemical parameters in lowland tropical tree species. Lin, Medlyn, and Ellsworth (2012) also reported that an increase in g_1 (the slope parameter of the optimal stomatal model, related to the marginal water cost of carbon to the plant) from 3.0 to 13.1 caused an increase in T_{opt} from 24.4 to

29.2 °C, and increase in A_{opt} from 14.8 to 22.7 μmol m^{-2} s^{-1}. These findings emphasize the need for incorporating information on the responses of g_s to environmental factors, such as changes in growth temperature and O_3, when studying the mechanisms on photosynthetic thermal acclimation in poplars.

No or small effects of warming on basal rates of photosynthetic capacity (i.e. V_{cmax}^{25} and/or J_{max}^{25}) were reported in many studies, for example, a meta-analysis of data from 36 species (Kattge & Knorr, 2007), a review of 22 different tree studies (Way & Oren, 2010), 141 C_3 species (Kumarathunge et al., 2019) and 9 tree species (Stefanski, Bermudez, Sendall, Montgomery, & Reich, 2020). In cases where warming has been found to affect the basal rates of photosynthetic capacity, the response to warming is usually correlated with the variations in leaf N and Rubisco (e.g. Way & Sage, 2008; Crous et al., 2018). Here we found no effects of warming on the ratios of V_{cmax}^{25}/N_{area} and J_{max}^{25}/N_{area}, suggesting that there was no re-allocation of leaf N to Rubisco carboxylation or regeneration, and that the activation state of Rubisco may not be affected by warming. EO$_3$ decreases leaf N allocation to photosynthesis, and increases leaf N allocation to cell walls to resist the possible damages by EO$_3$ (Shang, Xu, Dai, Yuan, & Feng, 2019). Accordingly, the reductions of V_{cmax}^{25} and J_{max}^{25} by EO$_3$ correlated with decreases in N_{area}, and N partitioning into photosynthesis (PNUE) and photosynthetic capacity (V_{cmax}^{25}/N_{area} and J$_{max}^{25}/N_{area}$). The positive relationships between N_{area} and photosynthetic capacity (V_{cmax}^{25} and J_{max}^{25}) (Figure S3) support these findings.

The temperature sensitivities of the photosynthetic capacity parameters were impacted by both warming and EO$_3$. Warming increased T_{optV} and K_{optV} but had no effects on T_{optJ}, K_{optJ} or on either of the activation energy terms (E_{aV} and E_{aJ}) (Figure 2; Table 2). These findings indicate differences in temperature sensitivity between RuBP carboxylation and RuBP regeneration as has been observed for several species previously (e.g. Farquhar & Caemmerer, 1982; Hikosaka, Murakami, & Hirose, 1999; Onoda, Hikosaka, & Hirose, 2005). However, thermal acclimation of V_{cmax} and J_{max} appears to vary among tree species. For example, warming caused a shift in T_{optJ}, E_{aJ} and E_{aV}, but not T_{optV} in *Picea mariana* and *Larix laricina* (Dusenge, Madhavji, & Way, 2020). Both V_{cmax} and J_{max} thermal acclimation occurred in 36 plant species resulting in an average T_{optV} and T_{optJ} increase by

0.44 °C and 0.33 °C per 1 °C increase of growth temperature (Kattge & Knorr, 2007). Overall, regardless of which of the capacity parameters acclimates, constructive thermal acclimation of photosynthetic capacity benefits A_{sat} in a higher growth temperature in any species compared to no acclimation. While we cannot infer the exact mechanism behind the thermal acclimation of the short-term temperature response of V_{cmax} from our results, several potential mechanisms have been suggested. These include a stimulation of enzymatic activity as well as a change in the thermal stability of the component membranes (Sage & Kubien, 2007), altered production of different Rubisco activase isoforms (Sage & Kubien, 2007; Yamori, Hikosaka, & Way, 2014), and/or the production of different Rubisco subunit isoforms (Hikosaka, Ishikawa, Borjigidai, Muller, & Onoda, 2006), and increases in mesophyll conductance (Evans & von Caemmerer, 2013). Although there is a strong positive correlation between N_{area} and photosynthetic capacity (V_{cmax} and J_{max}) (Figure S3), we note that temperature responses of photosynthetic capacity parameters were not affected by foliar *N* content *per se* in mature *Pinus sylvestris* (Tarvainen, Lutz, Räntfors, Näsholm, & Wallin, 2018). Taken together these previous findings suggest that any *N*-mediated thermal acclimation responses likely occur through changes in *N* partitioning, rather than in total leaf *N* content.

Elevated O_3 significantly decreased all of the parameters describing the temperature responses of V_{cmax} and J_{max}, including T_{optV} and T_{optJ}, K_{optV} and K_{optJ}, and E_{aV} and E_{aJ}. Furthermore, the positive effect of warming on K_{optV} was lost at EO_3 (Figure 2; Table 2). Strong links exist between E_{aV} and E_{aJ} and the thermal optimum of photosynthetic capacity (e.g. Medlyn et al., 2002; Smith & Dukes, 2017) and net photosynthesis (Hikosaka, Ishikawa, Borjigidai, Muller, & Onoda, 2006; Kumarathunge et al., 2019; Dusenge, Madhavji, & Way, 2020). Thus O_3-induced reductions in enzyme activity may explain the observed decreases in T_{optV} and T_{optJ}, and further the decrease in T_{optA} under EO_3. Because O_3 concentrations commonly peak during the summer when the solar irradiance and the air temperature are the highest, the decrease in photosynthetic temperature optima, especially the large observed reduction in T_{optJ}, may have significant long-term consequences for plant carbon uptake. Importantly, Kumarathunge et al. (2019) recently developed an algorithm that accounts for thermal acclimation of photosynthetic capacity to be incorporated into

terrestrial biosphere models. Our study found that EO$_3$ significantly alters the temperature sensitivity parameters of photosynthetic capacity (e.g. E_{aV} and E_{aJ}), suggesting that the functions may need to be further modified for use in areas with high O$_3$ concentrations.

The temperature response of respiration must be considered too, as mitochondrial respiration can affect the net photosynthetic rate without gross photosynthesis being altered (e.g. Atkin, Bruhn, & Tjoelker, 2005a; Atkin, Bruhn, Hurry, & Tjoelker, 2005b; Way & Yamori, 2014). Acclimation commonly results in a reduction of the long-term temperature sensitivity of respiration, resulting in similar overall respiration rates in contrasting thermal environments (Atkin & Tjoelker 2003). In our study, neither the values of R_{dark} and R_{dark}^{25} nor Q_{10} were affected by warming. EO$_3$ also had no effects on R_{dark}^{25} or Q_{10}, indicating that EO$_3$ did not affect the temperature-sensitivity of R_{dark} in the studied poplar saplings. However, the effects of EO$_3$ on R_{dark} differ among species. Some studies have reported that EO$_3$ stimulates R_{dark}, which reduces O$_3$-induced damage by enhancing certain metabolic processes (Barnes, 1972; Yang, Skelly, & Chevone, 1983). Whereas other studies have found that EO$_3$ decreases R_{dark} in response to changes in membrane permeability and mitochondrial structure (Zheng et al., 2012). Such variation in the observed R_{dark} response to EO$_3$ may be related to differences in the O$_3$ concentrations and fumigation times used in the studies, and to differences in other environmental factors during the experiments. Importantly, the fact that neither R_{dark}^{25} nor Q_{10} were affected by either warming or EO$_3$ in the current study, suggests that leaf respiration in poplar may not increase with continued warming or greater O$_3$ pollution. Such a homeostatic respiratory response would contribute towards improved photosynthetic efficiency (A_{sat} increases, R_{dark} is unchanged) at a given temperature regardless of O$_3$ damage, and also reduce carbon losses at high temperatures.

4.3 Limitations of the current study

As noted in the material and methods section, the current study did not include replication at the level of solardomes but rather used individual plants as the experimental unit. However, the concerns regarding this limitation may be reduced by the study design that utilized plants that were of similar initial size, and included rotating the treatments and the plants among the solardomes to avoid any bias caused by chamber effects. Another

limitation of the study is the low number of biological replicates (three) chosen so that all of the temperature response curve measurements could be finished in the shortest possible time to ensure similar cumulative AOT40 for plants exposed to a given treatment. We acknowledge that the low number of replicates results in lower statistical power and increases the risk for type II error. Future experiments with greater replication may thus be able to detect additional significant individual and interactive effects of warming and elevated O_3 on the acclimation of the photosynthetic temperatures responses.

The results presented in the current manuscript provide a first step towards including O_3 effects on the photosynthetic thermal acclimation capacity in global vegetation models and thus facilitate future model-based studies of acclimation and adaptation responses at larger spatial scales. Such model-based studies should then be compared to observed trends in natural stands and data from field exposure experiments to better understand the physiological underpinnings of acclimation and adaptation responses at the community/ecosystem level.

Conclusion

Overall, our study shows that both warming and elevated O_3 affect the thermal acclimation of photosynthesis in *Populus tremula*. Warming increased the thermal optimum of light-saturated net photosynthesis due to increases in stomatal conductance and the thermal optimum of the maximum rate of carboxylation, partially supporting hypothesis #1. However, the degree of photosynthetic thermal acclimation was reduced by elevated O_3 due to O_3-induced reductions in stomatal conductance, and in the temperature optima of V_{cmax} and J_{max}, supporting hypothesis #2. Furthermore, the responses of A_{sat}, V_{cmax} and J_{max} to warming and EO$_3$ were closely correlated with leaf N contents and N use efficiency. To our knowledge, the reduction of thermal acclimation capacities of A_{sat}, V_{cmax} and J_{max} by EO$_3$ has not been reported previously. Neither R_{dark} nor its temperature response were affected by warming or elevated O_3. This homeostatic response suggests potential for acclimation towards reduced carbon losses by respiration under heatwaves and periods of high O_3 concentrations, benefiting the survival of *Populus tremula* during environmental stress. Overall, our findings suggest that the photosynthetic thermal acclimation capacity may be

overestimated if O_3 exposure is not considered. Thus, accounting for the effects of both increasing growth temperatures and elevated O_3 on the temperature response of A_{sat} may help to refine the predictions of forest CO_2 exchange under climate change. These results may provide a starting point for inclusion of O_3 effects on photosynthetic thermal acclimation capacity in global vegetation models.

Author contributions

Z.F, H.H and L.D conceived the experiment. L.D conducted the experiment and collected the data. L.D, X.Y and L.T analysed the data. L.D, D.H, L.T, F.H and K.S made figures and tables. All the authors wrote and revised the manuscript and approved the final manuscript.

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